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ORIGINAL ARTICLE

Attenuation of Hydrogen Peroxide and Ferric Reducing/Antioxidant Power Serum Levels in Colorectal Cancer Patients with Intestinal Parasitic Infection

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Abstract

Background: **This study assessed several common oxidative indices in subjects infected with intestinal parasites, as well as in colorectal cancer (CRC) patients both with and without intestinal parasites.**

Method: **Serum levels of malondialdehyde (MDA), ferric reducing/antioxidant power (FRAP), and hydrogen peroxide (H_2O_2) were measured, as were plasma levels of advanced oxidation protein products (AOPP), all according to established methods. The presence of intestinal parasites was confirmed by stool examination.**

Results: **All intestinal parasiteinfected subjects and CRC patients showed the presence of oxidative stress. Thirtysix percent of the CRC patients had intestinal parasitic infections. The levels of H_2O_2 and FRAP in parasite-infected subjects were significantly higher than in CRC patients, but these levels were significantly lower in the CRC patients with parasitic infections.**

Conclusion: **Parasitic infection and CRC may contribute to oxidative stress independently, but when present together, the oxidative stress burden imposed by parasites may be attenuated.**

Keywords: *Colorectal neoplasm, parasites; oxidative stress, medical sciences*

Introduction

Protozoan and helminth parasites secrete enzymes that generate superoxides and reactive oxygen intermediates (such as hydrogen peroxide (H_2O_2)) in mammalian tissues (1). Reactive oxygen species (ROS) are produced by the imflammatory cells of the host, and these work to kill the invading parasites by nitration, oxidation and chlorination reactions (2). These reactive species have also been implicated as contributing factors in the pathophysiology of cancer (3) and various other diseases (4,5). Oxidative stress occurs as a result of a shift in the balance between the production of free radicals and antioxidant defenses (6) in favour of free radicals, specifically ROS. ROS such as superoxide radicals (O_2), hydroxyl radicals ($OH^{\cdot}$), H_2O_2 and hydroperoxide can inflict oxidative damage on lipids, proteins and nucleic acids (3). This can lead to DNA mutations, which in turn are carcinogenic (7).

The global prevalence of colorectal cancer (CRC) is high, and it accounts for 655,000 deaths out of 58 million deaths worldwide in 2005 (8). In Malaysia, CRC is the most common cancer among men (14.2% of male cancers) and the third most common cancer among women (10.1% of female cancers) (9). Factors such as age, diet, and genetic susceptibility have been suggested to contribute to the aetiology of CRC (10), and these risk factors may exert their effects through prolonged oxidative stress (3). Pathological records and analyses have shown hostand organ dependent correlations between numerous types of cancers and parasitic infections (11,12). However, assessments of the presence of parasites in CRC patients and their effect on oxidative stress are still lacking.

Therefore, in this study, the levels of various oxidative indices in CRC patients and parasite infected patients were compared.

Table 1: Demographic data of the study subjects

Variables	Normal	Subject Groups		
		CRC with parasitic infection	CRC without parasitic infection	Parasitic infection only
n	100	21	38	40
Age	57 ± 12	59 ± 11	57 ± 12	53 ± 15
Gender				
Male	43	12	20	26
Female	57	9	18	14
Chemotherapy Regimen	Nil	Mayo (fluorouracil, foliclinic acid) or Folfox (Folinic acid, Fluorouracil, Oxaliplatin)	Mayo (fluorouracil, folinic acid) or Folfox (Folinic acid, Flouroucacil, Oxaliplatin)	NA

Materials and Methods

Subjects

A total of 59 patients were recruited from the ncology Clinic at the University Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia. All the CRC patients were undergoing chemotherapy. Twenty-one of these patients were confirmed to be infected with one or more intestinal parasites by stool examination and were classified as CRC patients with parasitic infections. Subjects without CRC but positive for parasitic infection were recruited among outpatients attending the UMMC and patients attending medical camps in the Klang Valley, Malaysia. Healthy volunteers (controls, *n*=100) were recruited from the same area where the medical camps were held. The study was approved by the Medical Ethics committee of the UMMC in accordance with the declaration of Helsinki.

Sample collection and preparation

Blood, urine and stool samples were collected from all subjects. Blood samples were collected in plain tubes and EDTA tubes to obtain serum and plasma, respectively. The separated serum and plasma were used for the analysis of oxidative indices. Urine and stool samples were collected in sterile cytology containers. Urinalysis using the dipstick method (Combur Test UX, Roche Diagnostics) was carried out to test for the levels of glucose, bilirubin, nitrite, protein, ketone bodies, urobilinogen and blood. Urine samples that showed biochemical abnormalities were excluded from this study. Parasitological investigation was also carried out on all stool samples to screen for the following parasites: *Entamoeba histolytica, Giardia lamblia, Blastocystis hominis, Microsporidia sp., Dientamoeba fraglis, Ascaris lumbrocoides, Trichuris trichiura,* hookworm and *Taenia sp.* Patients that tested positive for one or more of these parasites were classified as parasite-infected subjects.

Oxidative indices

Serum was used for the assessment of lipid peroxidation (LP), ferric reducing/antioxidant power (FRAP) and H_2O_2, whereas plasma was used for the determination of advanced oxidation protein products (AOPP). LP was determined by measuring malondialdehyde (MDA) according to the modified method of Ratty and Das (12), using 1,1,3,3- tetraethoxypropane as a standard. The FRAP assay was carried out according to the method of Benzie and Strain (13). The reduction of ferric tripyridyltriazine (Fe^{III}TPTZ) complex by the non-enzymatic antioxidants in biological fluids was monitored spectrophotometrically using a kinetic mode at 593nm. Serum H_2O_2 was measured using the ferrous ion oxidative xylenol orange version-2 (FOX-2) method of Benarjee et al. (14). AOPP was measured spectrophotometrically according to the method of WitkoSarsat et al. (15).

Parasitological analysis

Fresh faecal samples were obtained from the subjects and preserved in 10% buffered formalin for parasitological investigation. Intestinal helminths: The formalinethyl acetate concentration (FEAc) method (16) was used to detect *Ascaris lumbrocoides, Trichuris trichuria,* hookworm and *Taenia sp.* Intestinal protozoa, *Dientamoeba fragilis* and *Giardia lamblia* were

Table 2: Non enzymatic oxidant/antioxidant indices in blood samples: Comparison of normal subjects, CRC patients with and without parasitic infections, and subjects with arasitic infections only

Sample Group	n	Oxidative indices			
		AOPP (μmol/L)	FRAP (μmol/L)	H_2O_2 (μmol/L)	MDA (μmol/L)
Normal	100	128.95±4.91	376.56±8.03	20.78±1.05	0.089±0.007
CRC with parasitic infection	21	192.41±85.21 **	370.53±80.15	25.07±3.27 **	0.125±0.071 *
CRC without parasitic infection	38	151.95±49.03 *	358.47±78.16	24.53±4.68 **	0.149±0.081 ***
Parasitic infection only	40	198.63±10.74 *** †	625.01±27.84 *** ## ††	41.90±2.26 *** ## ††	0.173±0.013 ***

Data are given as mean ± SEM
**P<0.001 * P<0.05, **P<0.01, comparison with controls using Student's t-test
Comparison among the diseased groups was analysed using ANOVA
##P<0.001 is the comparison between 'CRC with parasitic infection' and 'Parasitic infection only'
†P<0.01, ††P<0.001 is the comparison between 'CRC without parasitic infection' and 'Parasitic infection only'

screened using the trichrome staining method (17), whereas *Microsporidia sp.* and *Blastocystis hominis* were detected with a modified trichrome staining method (18) and a culture technique (19) respectively.

Statistical analysis

Data were analysed with SPSS for Windows (Version 13.0). All data are expressed as means ± SEM, and significant differences between the patient and control groups were analysed using Student's *t*-test. Comparisons of the measured parameters among the patient groups were analysed using ANOVA.

Results and Discussion

The demographic data of the CRC patients (with and without parasitic infections) and the parasite-infected subjects without CRC are presented in Table 1. All the cancer patients were on chemotherapy, and the one should not rule out the possible influence that different anti-cancer drugs may have on the levels of oxidative damage. However, when we made comparisons among the cancer patients according to the type of chemotherapy they were undergoing, no significant differences were observed (results not shown). The possibility remains that the different chemotherapy protocols affected the oxidative indices, but did so similarly.

Parasite-infected subjects without CRC were also found to be positive for multiple parasitic infections. Comparisons based on the type and multiplicity of the parasitic infection did not show significant differences (result not shown), although this could have been due to the small sample size. Interestingly, 18 CRC patients were positive for *Microsporidia* sp., one was positive for *Blastocystis hominis* and two were positive for both *Microsporidia* sp. and B*lastocystis hominis* (data not shown). In all, 36% (21/59) of the CRC patients tested positive for intestinal parasitic infections, and the most common infection was *Microsporidia* sp. an opportunistic parasite. Rudrapatna et al. (20) reported that only 16.5 % of 1029 cancer patients tested positive for intestinal parasitic infections. Botero et al. (21) showed that in immunocompromised patients, only 9% (10/111) had opportunistic parasitic infections such as *Cryptosporidium* sp., *Microsporidia* sp. and *Strongyloides stercoralis*. Conversely, 42% of Malaysian children with cancer were reported to be positive for intestinal parasites (22).

MDA levels, which are used as an indicator of oxidative stress in cells and tissues (23), reflect the

overall lipid damage level. Lipid damage is a well-known mechanism of cellular injury in humans. We found that serum MDA levels were higher in both CRC patients and parasite-infected subjects (P <0.001 for each) than in normal individuals (Table 2). Numerous studies have shown that MDA levels in both plasma and tumour tissues of CRC patients are elevated (24,25). Also, parasitic infections, especially intestinal parasites, are known to cause cellular damage (26). This could lead to the elevated serum MDA levels in patients with intestinal parasites such as Giardia sp. (27) and Blastocystis hominis (23). However, in the present study, the MDA levels among the three groups were not significantly different (Table 2).

Plasma and serum AOPP levels have been widely used as markers of free radical induced protein damage in renal disease (15,28), diabetic complications (29), patients with parasitic infections and patients with CRC (30,31). To the best of our knowledge, this is the first study that reports elevated plasma AOPP levels in CRC patients with simultaneous parasitic infections. The increased plasma AOPP levels in the CRC and parasite-infected patient groups indicate that these patients have a higher degree of oxidative protein damage than do healthy controls (Table 2). Similarly, Kosova et al. (32) showed that serum AOPP levels were significantly higher in thyroid cancer patients than in normal subjects. AOPP levels in subjects with parasitic infections only were significantly higher than they were in CRC patients without parasitic infection (P <0.01), but were comparable to CRC patients with parasitic infections (Table 2). An in vitro study of nematode parasites demonstrated the existence of an inflammatory system capable of generating hypohalous acids (33) such as HOCl. This could explain the enhanced formation of AOPP in the subjects infected with parasites.

H_2O_2 is an oxidizing agent that can easily be converted into $OH^{\cdot}$ when exposed to ultraviolet rays or ferrous ions (34). Urinary H_2O_2 has been used as an oxidative stress biomarker in malignancy (14) and urinary tract infections (35). The CRC patients and the parasite-infected subjects showed higher levels of H_2O_2 than the healthy controls (Table 2). Interestingly, the H_2O_2 levels in parasite-infected subjects were almost two-fold higher than in CRC patients, but were attenuated in parasite-infected subjects with CRC (Table 2). One possibility is that, in order to fight parasites, monocytes produce $O_2^{\cdot}$ by stimulating xanthine oxidase activity ($O_2^{\cdot}$ is formed when xanthine oxidase converts hypoxanthine to xanthine). This excess $O_2^{\cdot}$ is then converted to H_2O_2 by superoxide dismutase (SOD) (34). When a parasitic infection is present in CRC patients who are on chemotherapy, glutathione peroxidase/catalase (H_2O_2-inactivating enzymes) may be induced, leading to a net reduction in H_2O_2 levels. Chemotherapeutic drugs are generally cytotoxic, and mononuclear cells have been reported to respond to cytotoxicity by increasing glutathione peroxidase levels (36).

FRAP levels are indicative of the total amount of non-enzymatic antioxidants. These include lipid-soluble antioxidants such as vitamin E, vitamin A, and provitamin A (beta-carotene); and water-soluble antioxidants such as vitamin C, uric acid, bilirubin and glutathione. We found that FRAP levels were lower in CRC patients than in healthy subjects (Table 2), possibly due to sequestration by tumour cells and/or oxidants released by the chemotherapeutic agents. Saygili et al. (24) observed reduced plasma vitamin C levels in CRC patients. Interestingly, we found that FRAP levels in the subjects with parasitic infections only were 66% higher than in any of other patients groups (Table 2). At first glance, this result appears to be in conflict with the general notion that under oxidative stress, increased macromolecular damage is accompanied by a reduction in antioxidant levels (37). However, the present findings are supported by another study that demonstrated a significant increase in non-enzymatic antioxidants in the serum of mice infected with *Trichinella spiralis* (38). This increase in FRAP levels in the parasite-infected subjects might be caused by an increase in serum uric acid due to biochemical changes in the body that occur in response to the infection. In agreement with this hypothesis, plasma uric acid levels were found to be elevated in mice with late stage *Plasmodium vinckei* infections (39). Previous studies have reported that increased plasma uric acid may account for as much as 60% of FRAP activity (13), and uric acid may be the main antioxidant in birds (40).

The reduced FRAP level in CRC patients with parasitic infections could be a consequence of the regulation of xanthine oxidase (XO) activity. When a parasitic infection occurs, XO (the dehydrogenase form) converts xanthine (which is generated during superoxide radical production for parasite eradication) to uric acid (41). Since the CRC patients with parasitic infections were also on chemotherapy, XO may be inactivated or inhibited by the drugs, leading to reduced net uric acid production, which in turn is reflected in the attenated FRAP level. A previous study has shown that anti-cancer drugs are potent xanthine oxidase inhibitors (42).

In conclusion, this study provides evidence

that patients with both CRC and parasitic infections secrete significant amounts of serum and plasma metabolites derived from oxidative stress damage. The incidence of parasitic infections in CRC patients is high. Parasitic infection and CRC may contribute to oxidative stress independently, but when present together, the oxidative stress burden imposed by the parasites may be attenuated.

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